PHOSPHOROLYSIS OF tRNA *. CONFORMATION OF YEAST $tRNA^{Phe}_{HCl}$ AND THE RECOMBINED MOLECULES WITH 3′ AND 5′ HALVES

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1. Introduction

Two classes of conformations of tRNA in solution have been shown by studies [1–3] on the reactivity of tRNA with polynucleotide phosphorylase. These conformations are defined by their ability (S conformation) or inability (R conformation) to be phosphorolyzed by the enzyme. The phenomenon was generally observed with unfractionated tRNA isolated from *E. coli*, yeast, or rat liver, as well as many purified specific tRNA from *E. coli* or yeast. Recently, it has been shown that yeast $tRNA^{Phe}$ could be chemically split [4], next to the ribose bearing the Y base, and still be recognized by phenylalanine-tRNA synthetase, 70% of the initial charging activity being restored [5]. Such modified tRNA molecules enable us to pursue phosphorolysis studies, in particular on the structural requirement for the recognition of the R and S conformations discriminated by polynucleotide phosphorylase.

2. Materials and methods

The enzymes used were the same as described previously [2, 3]. Yeast $tRNA^{Phe}$ was purchased from Boehringer (Mannheim, Germany). DEAE-cellulose, type SN, capacity 0.48, was a product of Serva (Heidelberg, Germany). Phosphorolysis was carried out as previously described [1]. The incubation mixture for phosphorolysis contained: tris-HCl, 50 mM (pH 8.1); $MgCl_2$, 0.5 mM; ^{32}P-K_2HPO_4, 10 mM; $tRNA^{Phe}$ (or the halves), A_{260} = 5.0/ml; polynucleotide phosphorylase, 25 units/ml. The ^{32}P-nucleoside diphosphates were extracted by a procedure previously described [1], dried on planchets and counted in a thin-window, slow-counter.

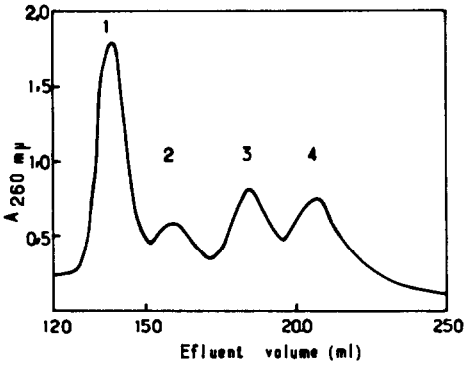

Fig. 1. Separation of the 3′- and 5′-halves of yeast $tRNA^{Phe}$ on DEAE-cellulose column. The mixture of the halves was chromatographed on DEAE-cellulose column (0.5 × 100 cm) previously equilibrated with 7 M urea (ph 3.0). Linear gradients of 200 ml each, 0.1 and 0.3 M NaCl in 7 M urea, adjusted with HCl to pH 3.0 was applied.

The acceptor activity of $tRNA^{Phe}$ and of the halves was determined as described by Thiebe and Zachau [4].

The excision of the Y base of yeast $tRNA^{Phe}$ and the subsequent splitting of $tRNA^{Phe}_{HCl}$ into two halves

* Parts I to III of this series are given in refs. [1–3].
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Table 1
Phenylalanine acceptor activity of $tRNA^{Phe}$, $tRNA^{Phe}_{HCl}$, and the halves of $tRNA^{Phe}$.

	cpm/A_{260}	Acceptor activity (%)	
		of $tRNA^{Phe}$ as 100%	of $tRNA^{Phe}_{HCl}$ as 100%
$tRNA^{Phe}$	257,700	100	-
$tRNA^{Phe}_{HCl}$	165,200	64	100
peak 1(3′-half)	4,800	1,8	2.9
peak 3(5′-half)	5,000	1,9	3.0
peak 4(5′-half)	4,200	1,6	2.5
peak 1 + peak 3	90,000	35	54.5
peak 1 + peak 4	64,000	25	33

Acceptor activity was determined as described by Thiebe and Zachau. The incubation mixture (0.1 ml) contains: 0.05 A_{260} units of $tRNA^{Phe}$ (or of the halves); 0.012 A_{280} units of aminoacyl-tRNA synthetase fraction; 1 μmole ATP; 1.5 μmoles $MgCl_2$; 2.5 μmole tris-HCl (pH 7.5); and 0.01 μmole ^{14}C-L-phenylalanine. Incubation at 25° for 40 min.

were carried out as described by Zachau et al. [4–6]. The separation of the halves of yeast $tRNA^{Phe}$ was performed on a DEAE-cellulose column (0.5 × 100 cm) with linear gradients of 200 ml each, 0.1 and 0.3 M NaCl in 7 M urea, adjusted with HCl to pH 3.0. Fractions of 3.8 ml/hr were collected.

3. Results and discussion

3.1. *Separation and acceptor activity of the halves*

Fig. 1. shows the pattern of separation of the halves of yeast $tRNA^{Phe}$; it is slightly different from that obtained by Wintermeyer et al. [7]: peaks 1, 3 and 4 correspond to the peaks 1, 2 and 3 found by these authors; peak 1 is the 3′-half, and peaks 3 and 4 the 5′-halves of the molecule. Peak 2 is probably also the 5′-half which might have the ribose moeity bearing the Y base modified in a different way, because the activity is not restored when it is combined with peak 1. The restoration of acceptor activity after recombination of peaks 1, 3 and 4 is presented in table 1. The peaks alone have traces of activity, and after recombination of peaks 1 and 3, and 1 and 4, 54% and 33% acceptor activity were obtained respectively as compared to the acceptor activity of $tRNA^{Phe}_{HCl}$ taken as 100%. In our hands $tRNA^{Phe}_{HCl}$ has 60–80% of the initial acceptor activity of $tRNA^{Phe}$.

3.2. *Phosphorolysis*

A variety of modified yeast $tRNA^{Phe}$ was available for the studies of phosphorolysis: 1) a tRNA with the Y base excised at the anticodon loop which was called $tRNA^{Phe}_{HCl}$; 2) a portion of tRNA with a PO_4 at the 5′ end and free OH groups at the 3′ end, which we call 3′-half; 3) a portion of tRNA with a PO_4 at the 5′ end but where the 3′ end does not have either the Y base nor the 3′ OH group, because aniline treatment modified the ribose moeity, yielding unknown derivatives, which we call the 5′-half; 4) a tRNA with the Y base excised and the internucleotide linkage adjacent to this base broken, which we call the recombined biological active molecule from 5′ + 3′-halves.

The phosphorolysis was assayed at three temperatures: 10°, 25°, and 31°. The first remarkable result was the behavior of the recombined molecule (fig. 2). The profile of the kinetic curves at those three temperatures is similar to that of the intact $tRNA^{Phe}$ molecule or the $tRNA^{Phe}_{HCl}$ molecule. The extent of phosphorolysis increases with the temperature. On the contrary, the 3′-half was phosphorolyzed in a linear manner and went to completion, even at 10°. The phosphorolysis of the 5′-half was more complicated. No data on a model polynucleotide with such a derivative at the 3'-end is available. The phosphorolysis profile of this molecule at 10° looks quite similar to that of the intact molecules. At 25° and 31°, both the rates and the extent of phosphorolysis are

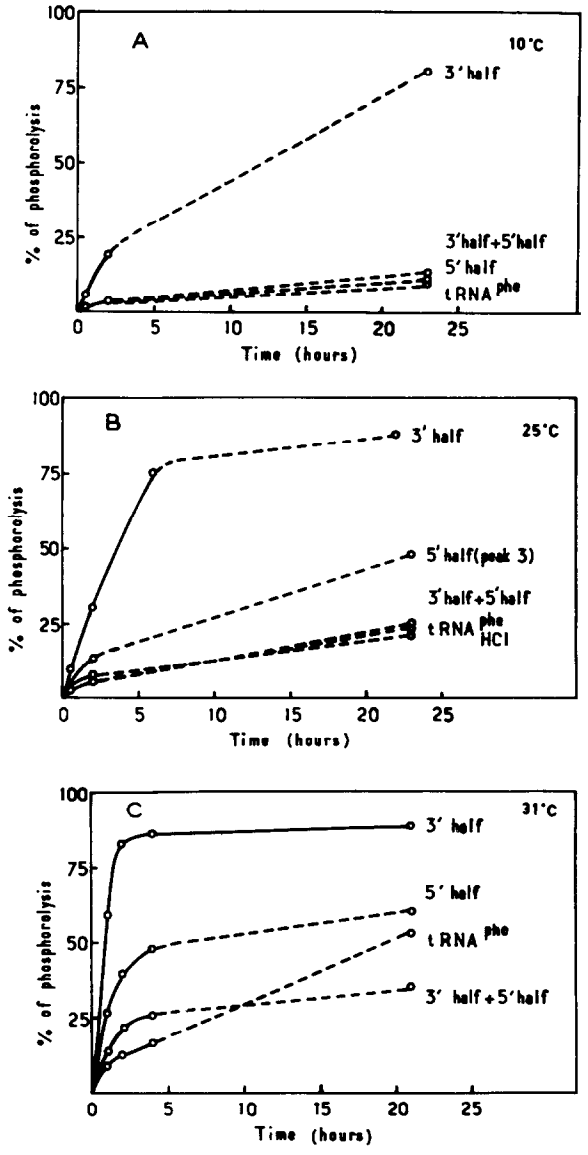

Fig. 2. Phosphorolysis of the halves of yeast $tRNA^{Phe}$ alone and when mixed. Phosphorolysis conditions as described in Materials and methods. A) Phosphorolysis at 10°; B) Phosphorolysis at 25°; C) Phosphorolysis at 31°.

higher. However, the kinetic curves are characterized by a rapid first phase, followed by a slow second phase.

These results might be interpreted in the following way: 1) The phosphorolysis of the 3′-half clearly indicates that this portion of the $tRNA^{Phe}$ behaves as a simple copolymer. It has been shown [8] by thermodynamic and kinetic studies that the helix-coil transitions in these 3′- or 5′-halves are cooperative processes, in which only fully base-paired and single stranded molecules occur in measurable concentrations. Thus, in such a system, no termination of phosphorolysis occurs, as can be expected.

2) The behavior of the $tRNA^{Phe}_{HCl}$ or of the recombined molecule toward polynucleotide phosphorylase is identical to that of the intact $tRNA^{Phe}$. Consequently, such molecules contain two classes of conformations, differentiated by their ability or inability to be phosphorolyzed. In other words, these molecules can assume in solution the R and S conformations, previously defined. The fact that the 3′-half can be degraded to completion at 10° implies that in the phosphorolysis of the recombined molecule, the enzyme does not interact individually with the 3′-half or the 5′-half. Because, if this were the case, one would expect to obtain at least 50% of phosphorolysis at all temperatures. Hence, the mixture of those two halves gives rise to a molecule recombined as a whole, as was postulated [8]. The reformed molecule behaves as the original tRNA not only towards phenylalanine-tRNA synthetase, but also toward polynucleotide phosphorylase. This suggests that modification brought on the anticodon loop, such as excising the Y base and introducing one nick on the phosphate linkage at this place, does not affect the molecules insofar as assuming the R and S conformations as well as their transition by temperature shifts.

3) Interpretation of the phosphorolysis of the 5′-half is more difficult. Because of the presence, at the 3′ end, of the modified ribose moeity derivatives and the assumption that the 5′-half has the same helix-coil transition process as the 3′-half, one should expect three possibilities for the phosphorolysis: a) the enzyme does not attack such molecules at all; b) the enzyme can degrade such molecules; c) the enzyme degrades one part of the molecules and not others, according to the chemical nature of the derivatives of the modified ribose moeity. But in this last case, the percentage of degraded molecules is not dependent on the temperature of the reaction. In fact, the experimental results show that the phosphorolysis is not an all or none phenomenon. There is partial phosphorolysis, but the extents increase with increasing temperatures. In the absence of precise information on the nature of the terminal derivatives, and on the mode of action of the enzyme on such mole-

cules, it is premature to discuss whether the phenomenon observed is related to the structure of the substrate or to the mechanism of the enzyme.

4. Conclusion

The recombined molecule made up with 3′- and 5′-halves of yeast $tRNA^{Phe}_{HCl}$ still contains the structural requirement for the recognition of the two classes of R and S conformations by the enzyme. Hence, it seems that the integrity of the anticodon loop is not necessary for the discrimination of these conformations by the enzyme. In addition, our experiments on the separation and the recombination of $tRNA^{Phe}$ are in good agreement with results obtained in Zachau's laboratory.

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